For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-CD43 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D056-3	1D4	Mouse IgG2a	100 µL	1 mg/mL

BACKGROUND: CD43 antigen is a heavily sialylated single chain membrane glycoprotein with both molecular weight and antigenicity depending on the nature and complexity of the attached O-linked polysaccharides. CD43 is an integral membrane protein with extracellular domain (235 aa), transmembrane domain (23 aa) and intracytoplasmic domain (123 aa). The extracellular domain of CD43 has been shown to react with many ligands such as ICAM-1 (CD54), E-selectin, galectin-1, MHC-I and human serum albumin (HSA). The intracytoplasmic domain, which is highly conserved between species, is constitutively phosphorylated on serine residues. The level of phosphorylation increases after cell activation. This cell surface glycoprotein is also called leukosialin, sialophorin or GPL115. A soluble form of CD43 is also present in human serum. Thymocytes, $CD4^+$ T lymphocytes and monocytes express more of a 115 kDa CD43 isoform, whereas a 130 kDa isoform is found mostly on activated $CD4^+$ T cells, $CD8^+$ resting and activated T cells, neutrophils, platelets and B cells. CD43 plays a role in the regulation of lymphoid cell adhesion, cellular maturation and activation although the nature of this participation remains controversial. For example, the structural characteristics of the CD43 extracellular domain indicate that this molecule has both adhesive and anti-adhesive properties. O-glycosylated chains of CD43 such as a sialyl Lewis X (sLe_x) epitope, a ligand for both P- and E-selectin, potentiate binding to lectin-like molecules. However, the adhesion molecule function for CD43 is counteracted by the presence of negatively charged sialic acid residues on its extracellular domain. In addition to the strong structural evidence for the involvement of CD43 in cell interactions, experimental data suggest contradictive role for CD43 in regulating cell contacts.

SOURCE: This antibody was purified from hybridoma (clone 1D4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with PHA-activated human T cell line maintained with IL-2 containing media.

FORMULATION: PBS containing 50% Glycerol (pH 7.2). No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with CD43 antigen on Flow cytometry. Clone 1D4 selectively recognizes core 2-containing *O*-glycans on CD43, which have the GlcNAcβ1 → 6 branch attached to the C-6 position of GalNAc and are formed by core 2 β1,6-N-acetylglucosaminyltransferase (C2GnT). This type of CD43 is preferentially expressed in the $CD45RO^+$ memory subset of $CD4^+$ cells.

APPLICATION-CONFIRMED:

Flow cytometry	5 µg/mL (final concentration)

APPLICATIONS-REPORTED:

Western blotting	Reference 3)
Immunohistochemistry	Reference 2)
Immunoprecipitation	Reference 1) and 5)

SPECIES CROSS REACTIVITY on FCM:

Species	Human	Mouse	Rat
Cells	lymphocyte, monocyte, granulocyte	Not Tested	Not Tested
Reactivity	+		

Entrez Gene ID:

6693 (Human)

REFERENCES:

1) Hernandez, J. D., *et al., J. Immunol.* **177**, 5328-5336 (2006) [IP]
2) Cabrera, P. V., *et al., Blood* **108**, 2399-2406 (2006) [IHC]
3) Fuhlbrigge, R. C., *et al., Blood* **107**, 1421-1426 (2006) [WB]
4) Mukasa, R., *et al., Immunol. Lett.* **67**, 117-124 (1999) [FCM]
5) Mukasa, R., *et al., Int. Immunol.* **11**, 259-268 (1999) [FCM, IP]

Clone 1D4 is used in these references.

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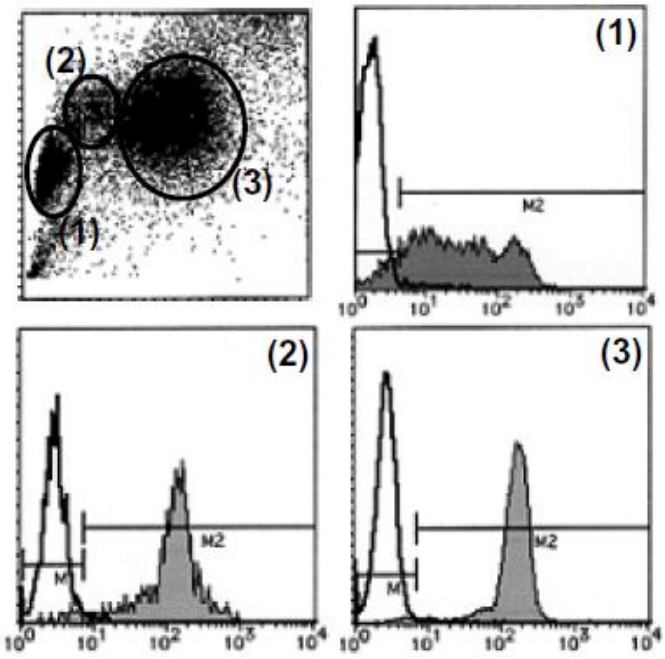

Flow cytometric analysis of CD43 antigen expression on lymphocyte (1), monocyte (2) and granulocyte (3). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D056-3 to the cells.*

PROTOCOLS:

Flow cytometric analysis for whole blood cells

We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.

1) Add 50 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 min. at room temperature (20~25°C).
3) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
4) Add 30 µL of 1:100 anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) diluted with the washing buffer. Mix well and incubate for 15 min. at room temperature.
5) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove incubate for 30 min. at room temperature.
6) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments, Beckman Coulter; code no. A11895) or OptiLyse B (for analysis on BD instruments, Beckman Coulter; code no. IM-1400) using the procedure recommended in the respective package inserts.
7) Add 1 mL of H_2O to each tube and incubate for 10 min. at room temperature.
8) Centrifuge at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Wash the cells 3 times with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
2) Resuspend the cells with the washing buffer (5 x 10^6 cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 min. at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well, and incubate for 5 min. at room temperature.
5) Add 40 µL of the primary antibody at the concentration of as suggested in the **APPLICATIONS** diluted in the washing buffer incubate for 30 min. at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
7) Add 30 µL of 1:40 anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) diluted with the washing buffer. Mix well and incubate for 15 min. at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; U937)

RELATED PRODUCTS:

D056-4	Anti-CD43 (Human) mAb-FITC
D056-5	Anti-CD43 (Human) mAb-PE
D119-3	Anti-CD43 (Mouse) mAb
D064-3	Anti-CD4 (Human) mAb
D064-4	Anti-CD4 (Human) mAb-FITC
K0003-1	Anti-CD4 (Human) mAb
K0006-1	Anti-CD8 (Human) mAb
D271-4	Anti-CD8 (Mouse) mAb-FITC
K0015-3	Anti-CD45RO (Human) mAb
K0015-4	Anti-CD45RO (Human) mAb-FITC

Isotype control

M076-3	Mouse IgG2a (isotype control)
M076-4	Mouse IgG2a (isotype control)-FITC
M076-5	Mouse IgG2a (isotype control)-PE

Other related antibodies and kits are also available.
Please visit our website at http://ruo.mbl.co.jp/